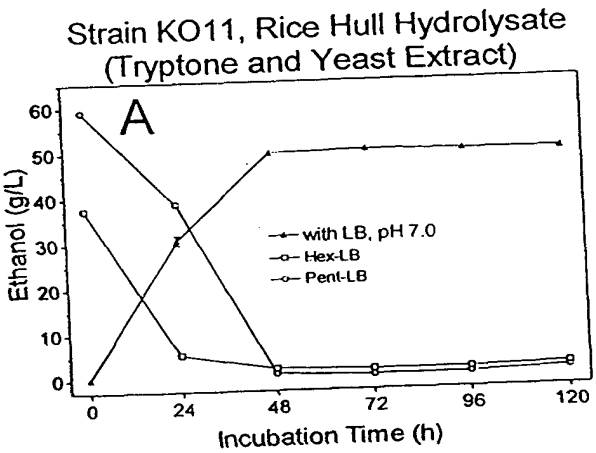
Fig. 1


Strain KO11, Rice Hull Hydrolysate
(Tryptone and Yeast Extract)
A
Ethanol (g/L)
Incubation Time (h)
with LB, pH 7.0
Hex-LB
Pent-LB
0
10
20
30
40
50
60
0
24
48
72
96
120

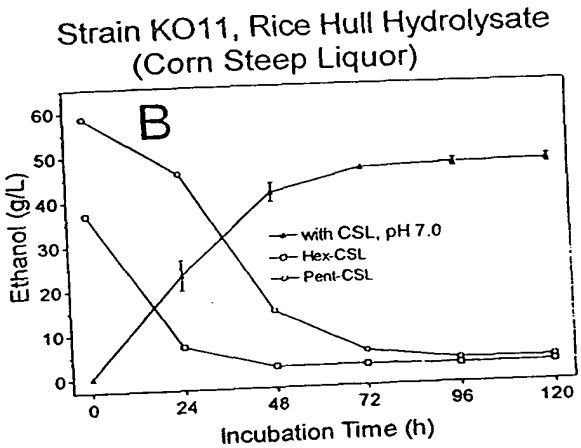

Strain KO11, Rice Hull Hydrolysate
(Corn Steep Liquor)
B
Ethanol (g/L)
Incubation Time (h)
with CSL, pH 7.0
Hex-CSL
Pent-CSL
0
10
20
30
40
50
60
0
24
48
72
96
120

Fig. 2

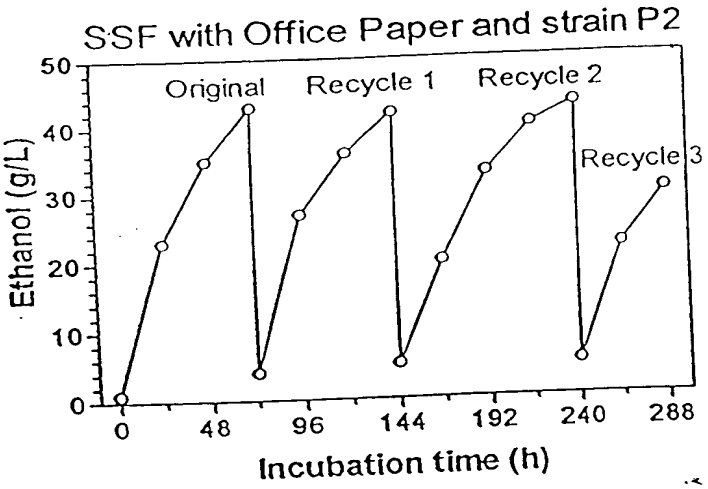
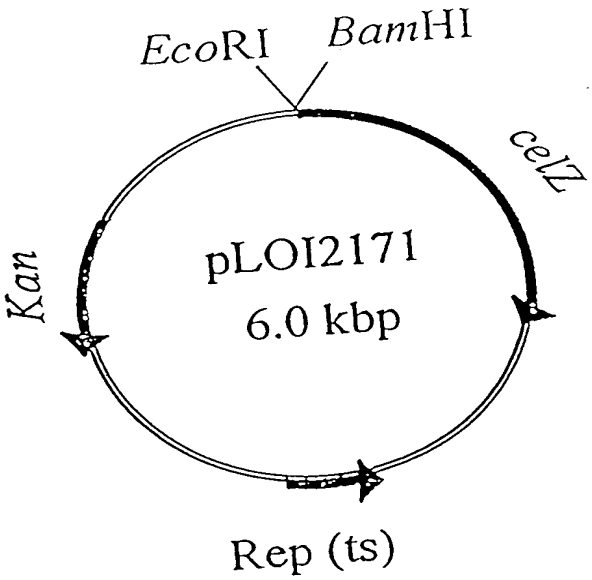

SSF with Office Paper and strain P2
Original
Recycle 1
Recycle 2
Recycle 3
Ethanol (g/L)
0
10
20
30
40
50
0
48
96
144
192
240
288
Incubation time (h)

Fig. 3



EP 0 417 903 A2

Fig. 4

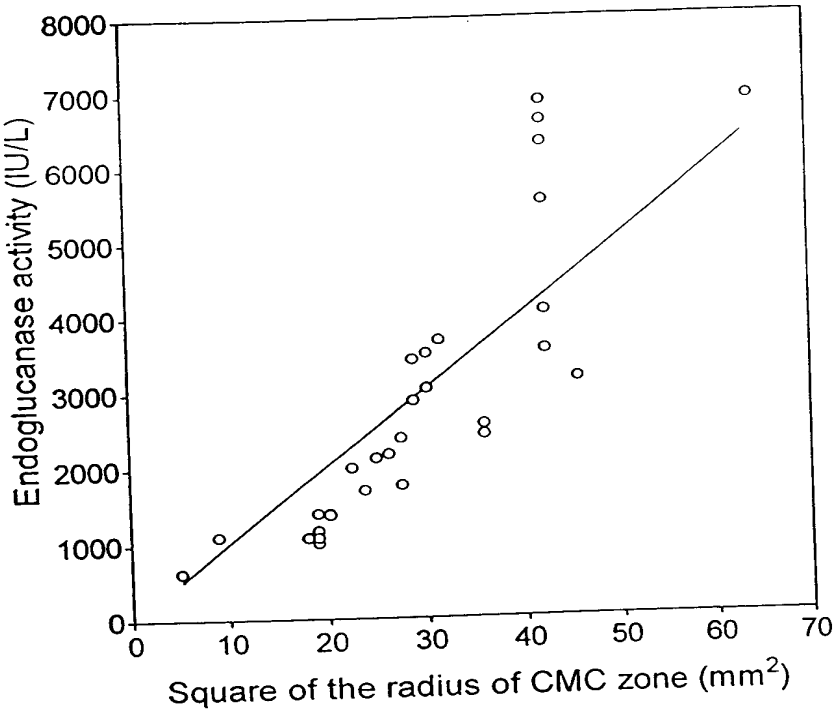

8000
7000
6000
5000
4000
3000
2000
1000
0
Endoglucanase activity (IU/L)
0
10
20
30
40
50
60
70
Square of the radius of CMC zone (mm2)

Fig. 5

```
                         -35 region                                 -10 region          #
1051 CTTTTTCGGC ATGAGCAACC  AACATTTTCA AGGTATCATC CTGATGCGCA

1101 ATATCGGCAT  CGGTTAGCCA TAACCATTTT  ACCTGTCCGG CGGCCTTAAT
1151 ACCTTGATCA  GATGGTTCGT  GGTGTTGTTA  CCTTGCCGAA  GGGCACCGGT
1201 AAAAATGTTC  GCGTCGGTGT  TTTCGCCCGT  GGCCCGAAAG CTGAAGAAGC
1251 TAAAGCTGCT GGTGCAGAAG  TTGTCGGCGC  AGAAGACCTG  ATGGAAGCCA

                                 -35 region                         -10 region
1301 TTCAGGGCGG CAGCATTGAT TTCGATCGTG ATGCCCTTTA TACTGAAATT

     #
1351 GCCTTGCGCT GCCATAATGA AGCAGCCTCC GGTGTTTTGG CAGATTTAAG

                                                   Shine-Dalgarno
1401 CGCTGCCTGA TTTTCGTgat cctctagagt ctatgaaatg gagattcatt

          celZ coding region-->
1451 tatgcctctc tcttattcgg ataaccatcc agtcatccgc aagcttggcc
```

Fig. 6

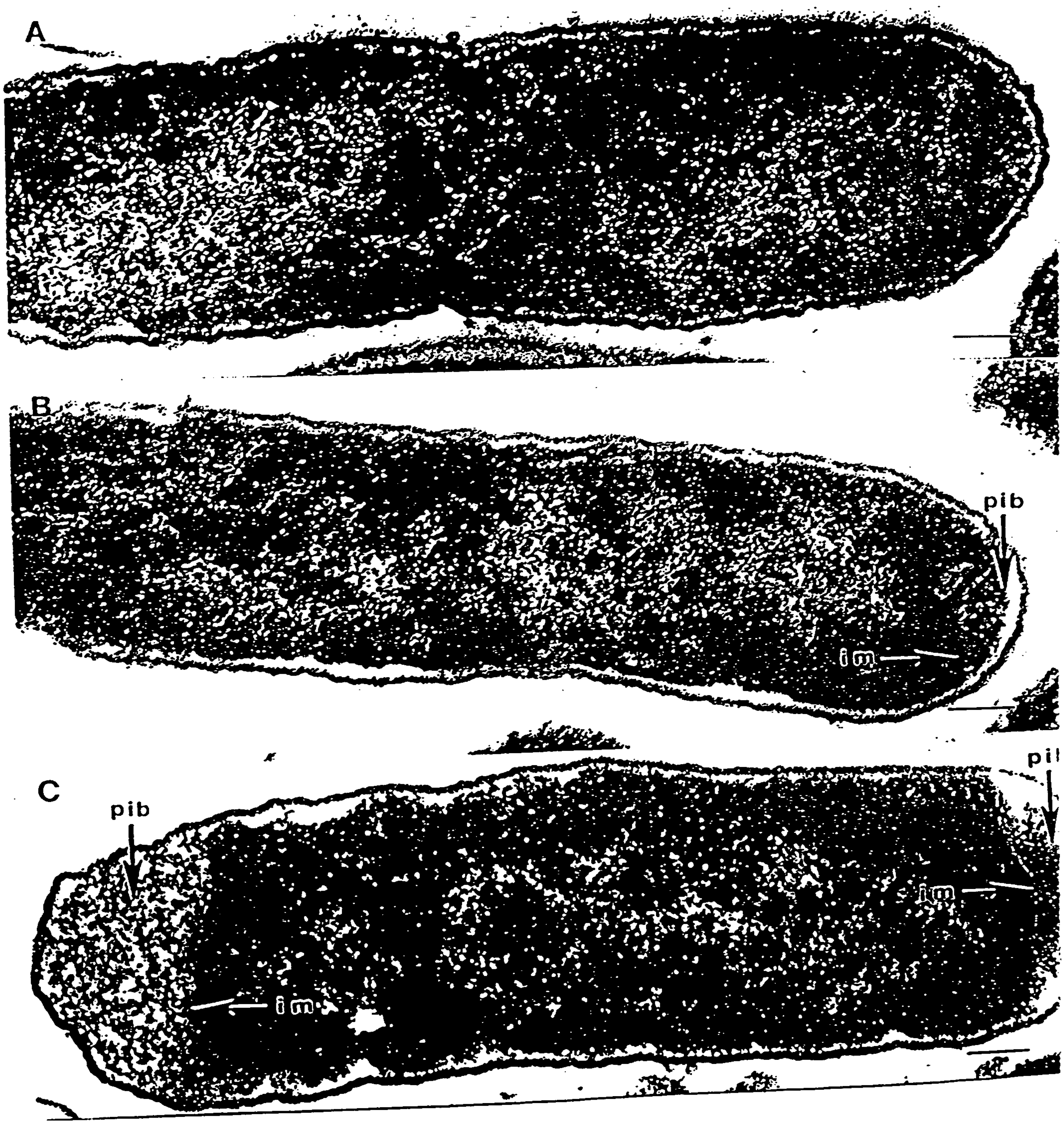


Fig. 7

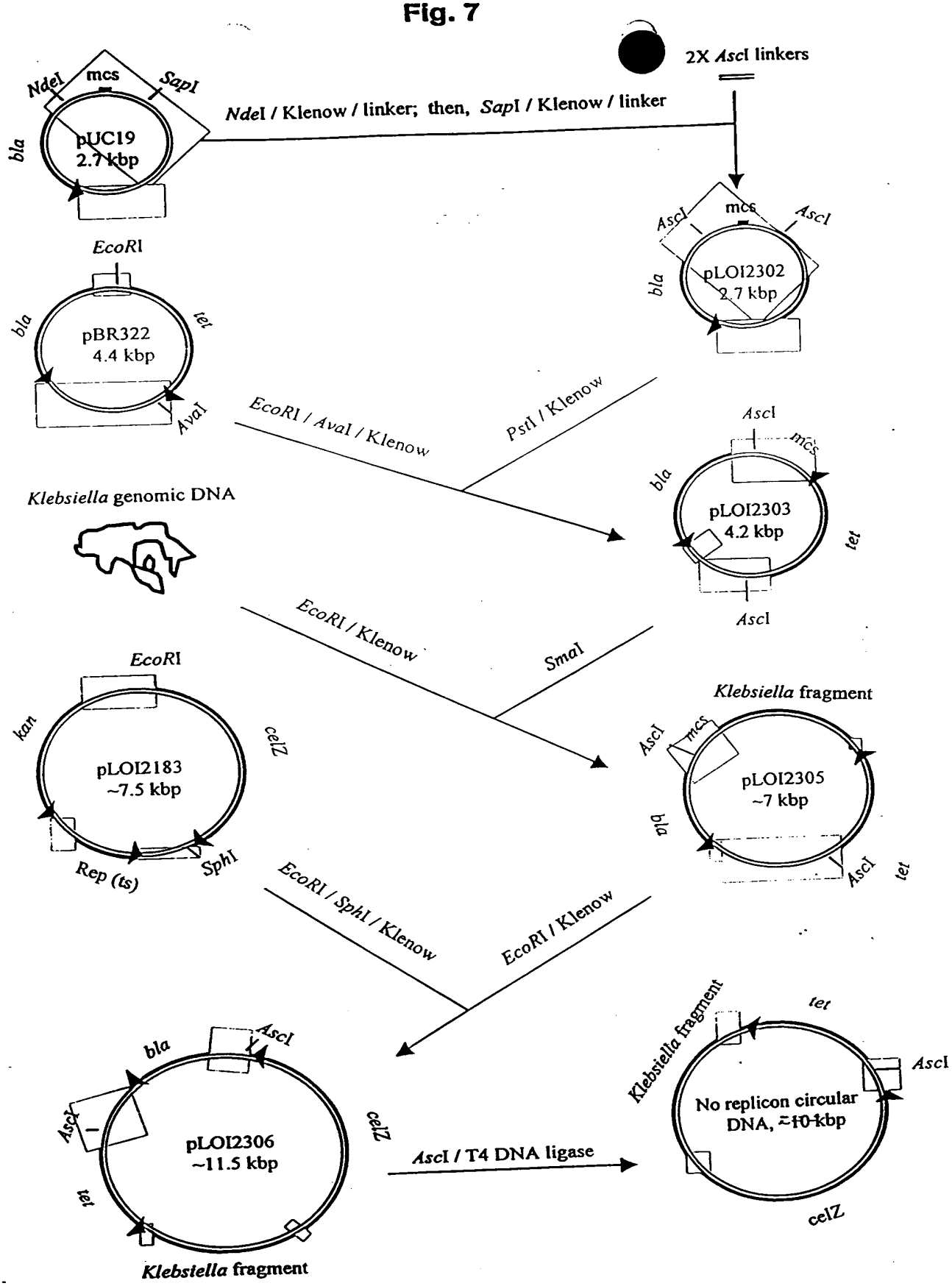
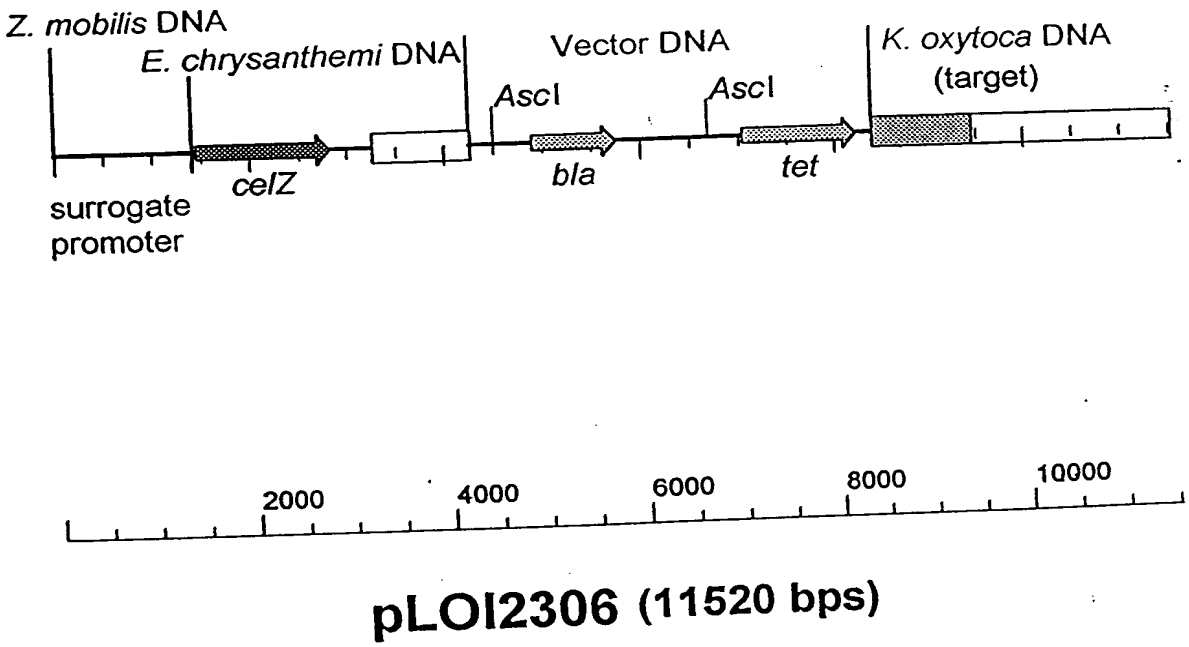
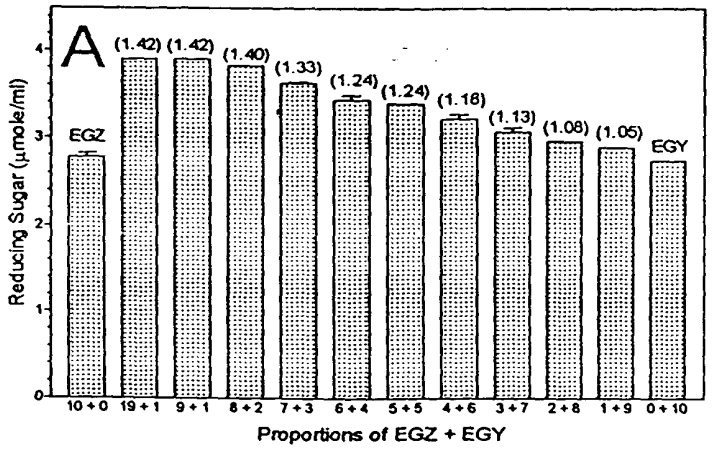

2X AscI linkers
NdeI / Klenow / linker; then, SapI / Klenow / linker
NdeI
mcs
SapI
bla
pUC19
2.7 kbp
AscI
mcs
AscI
bla
pLOI2302
2.7 kbp
EcoRI
bla
pBR322
4.4 kbp
tet
AvaI
EcoRI / AvaI / Klenow
PstI / Klenow
AscI
mcs
bla
pLOI2303
4.2 kbp
tet
AscI
Klebsiella genomic DNA
EcoRI / Klenow
SmaI
EcoRI
kan
pLOI2183
~7.5 kbp
celZ
Rep (ts)
SphI
Klebsiella fragment
AscI
mcs
pLOI2305
~7 kbp
bla
AscI
tet
EcoRI / SphI / Klenow
EcoRI / Klenow
bla
AscI
AscI
pLOI2306
~11.5 kbp
celZ
tet
Klebsiella fragment
AscI / T4 DNA ligase
Klebsiella fragment
tet
AscI
No replicon circular DNA, ~10 kbp
celZ

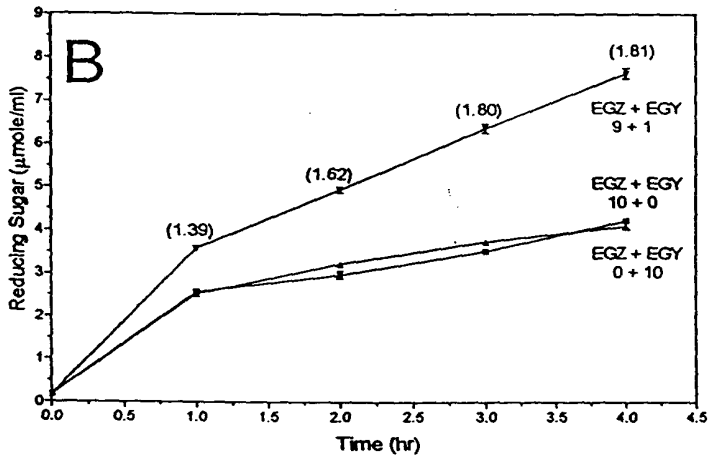
Fig. 8

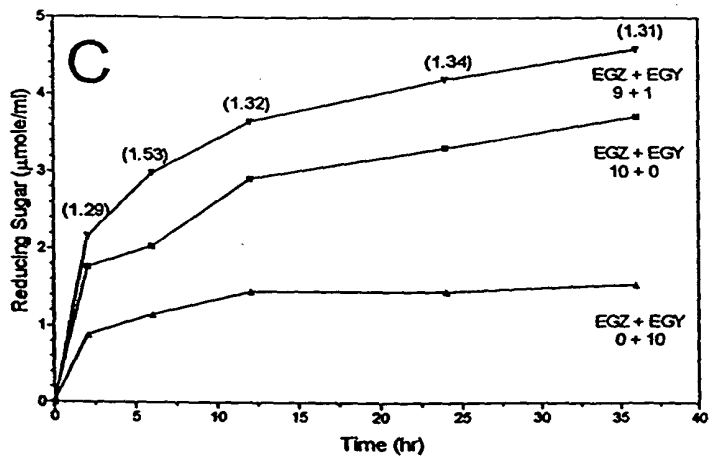


pLOI2306 (11520 bps)

Fig. 9







US 6,569,657 B1

Fig. 10

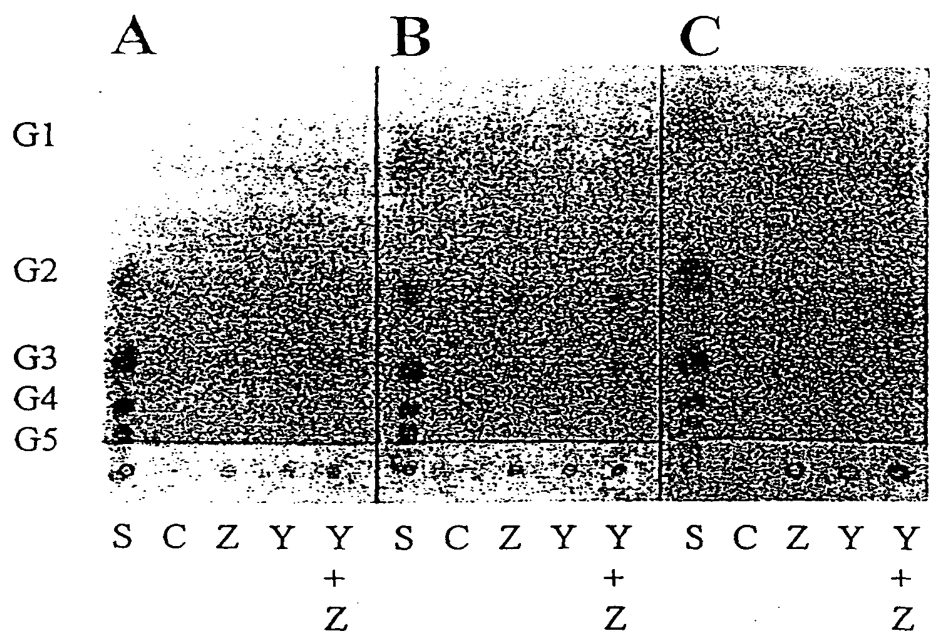


Fig. 11

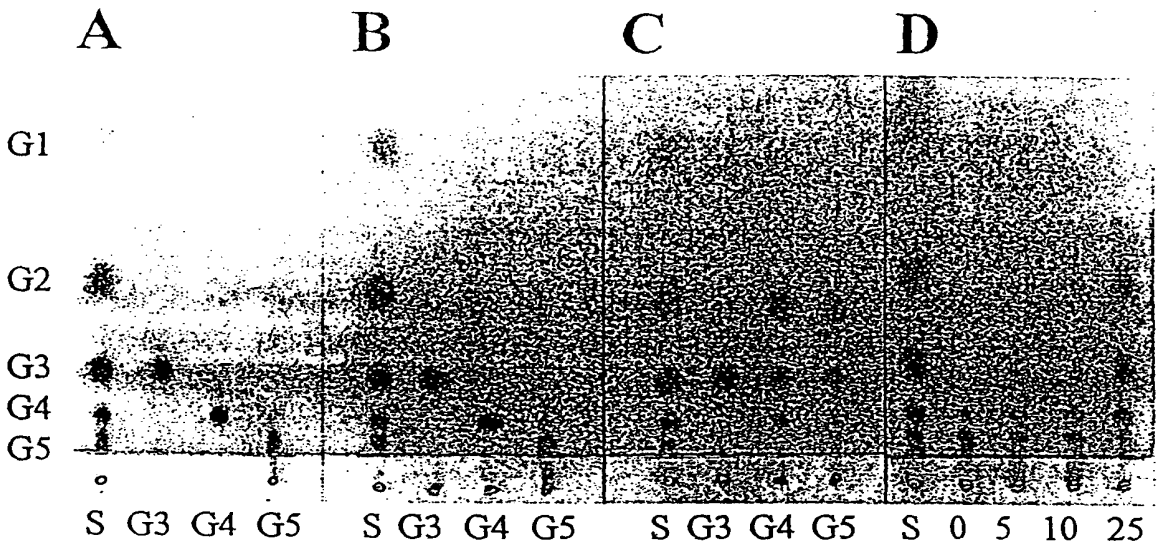


Fig. 12

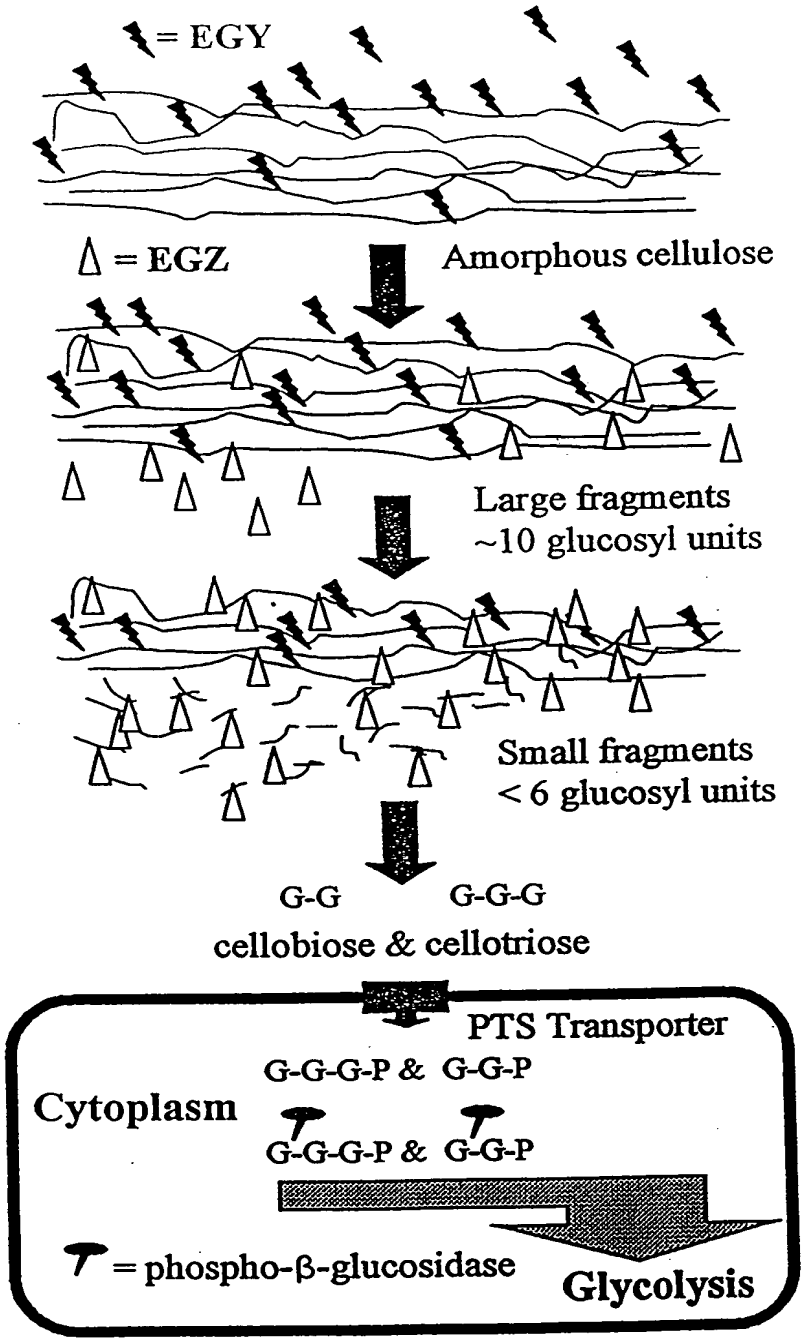
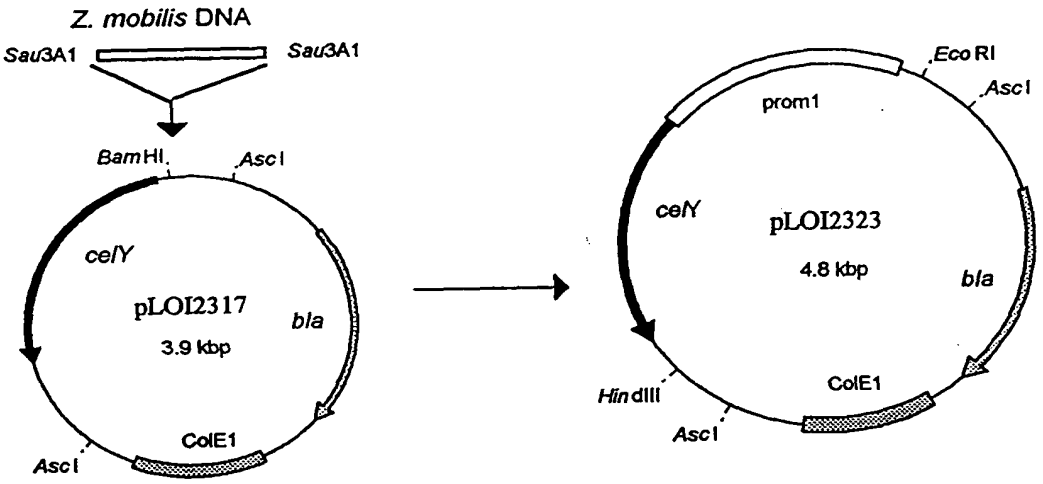

= EGY
= EGZ
Amorphous cellulose
Large fragments
~10 glucosyl units
Small fragments
< 6 glucosyl units
G-G
G-G-G
cellobiose & cellotriose
PTS Transporter
G-G-G-P & G-G-P
Cytoplasm
G-G-G-P & G-G-P
= phospho-β-glucosidase
Glycolysis

Fig. 13


Z. mobilis DNA
Sau3A1
Sau3A1
BamHI
AscI
celY
pLOI2317
3.9 kbp
bla
ColE1
AscI
EcoRI
AscI
prom1
celY
pLOI2323
4.8 kbp
bla
ColE1
HindIII
AscI

T06T90" 26259860

Position (bp)	-35	-10	RNA Start	Proposed δ factors	δ factor consensus sequence	
					-35	-10
	ATATTTTTGATTTTCAAGAAAAGCCTGATATCTTCCAACATCTT		**T** (2)	δ^{70}	TTGACA	TATAAT
	GATTTGATCCTCTAGAGTCAACCTGCTTGTTACTCGTGATCCCAT		**A** (4)	δ^{70}	TTGACA	TATAAT
	GAGTCAACCTGCTTGTTACTCGTGATCCCATTCACAAGGGCGAA		**C** (1)	δ^{32}	CTTGAAA	CCCCAT
	TTACTCGTGATCCCATTCACAAGGGCGAATTAATTCGCCCTT		**C** (3)	δ^{38}	CCGCCT	TATACT

[a] Transcriptional starts for *celY* were identified by primer extension analysis. Four promoters were identified. Upstream sequence of these promoters with similarity to *E. coli* -35 and -10 regions are marked with underlines. RNA start sites are bolded. Putative promoters are numbered in parenthesis adjacent to the start site in decending order from the strongest. Differences in intensities were small, within 2-fold.

Fig. 14

Fig. 15

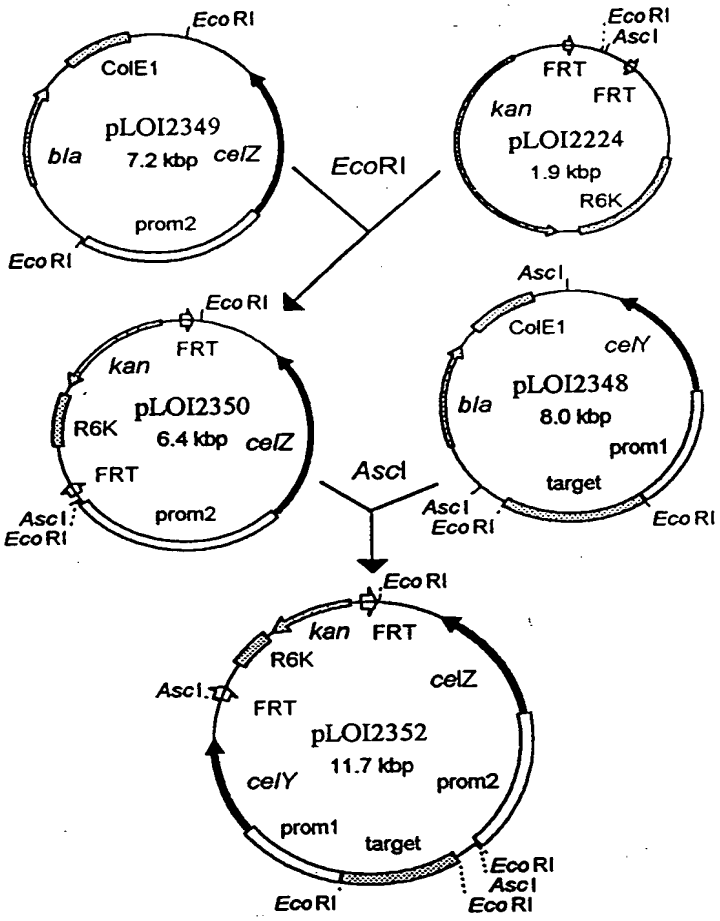

EcoRI
ColE1
pLOI2349
bla
7.2 kbp
celZ
prom2
EcoRI
EcoRI
AscI
FRT
FRT
kan
pLOI2224
1.9 kbp
R6K
EcoRI
AscI
ColE1
celY
pLOI2348
bla
8.0 kbp
prom1
target
AscI
EcoRI
EcoRI
EcoRI
FRT
kan
pLOI2350
R6K
6.4 kbp
celZ
FRT
AscI
EcoRI
prom2
AscI
EcoRI
kan
FRT
R6K
celZ
AscI
FRT
pLOI2352
11.7 kbp
celY
prom2
prom1
target
EcoRI
AscI
EcoRI
EcoRI

Fig. 16

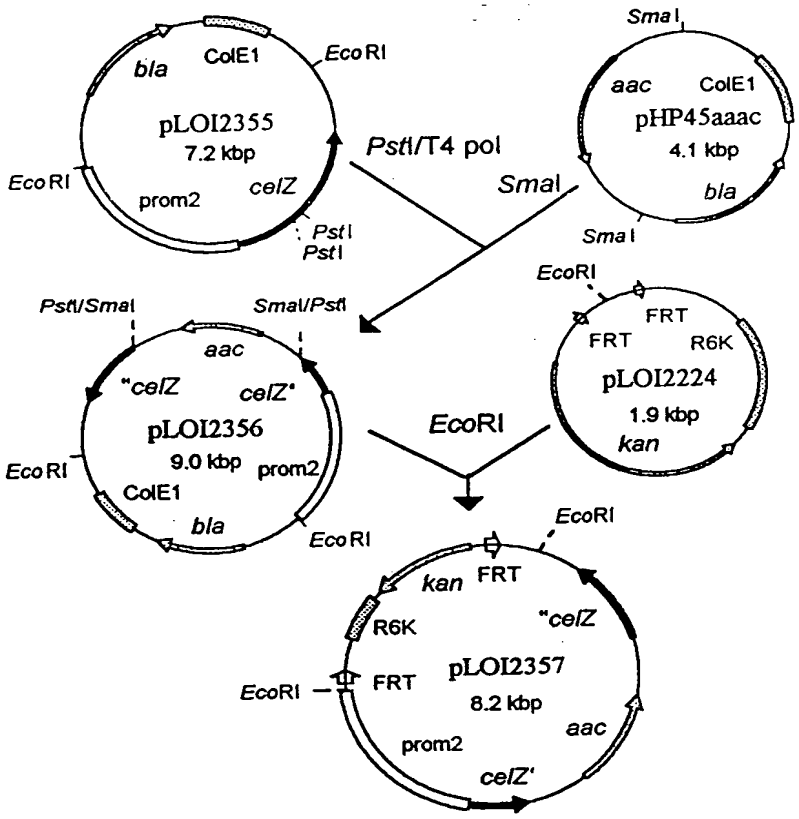

bla
ColE1
EcoRI
pLOI2355
7.2 kbp
EcoRI
prom2
celZ
PstI
PstI
PstI/T4 pol
SmaI
SmaI
aac
ColE1
pHP45aaac
4.1 kbp
bla
SmaI
EcoRI
FRT
FRT
R6K
pLOI2224
1.9 kbp
kan
PstI/SmaI
SmaI/PstI
aac
"celZ
celZ"
pLOI2356
9.0 kbp
prom2
EcoRI
ColE1
bla
EcoRI
EcoRI
kan
FRT
R6K
"celZ
pLOI2357
8.2 kbp
EcoRI
FRT
aac
prom2
celZ'

Fig. 17

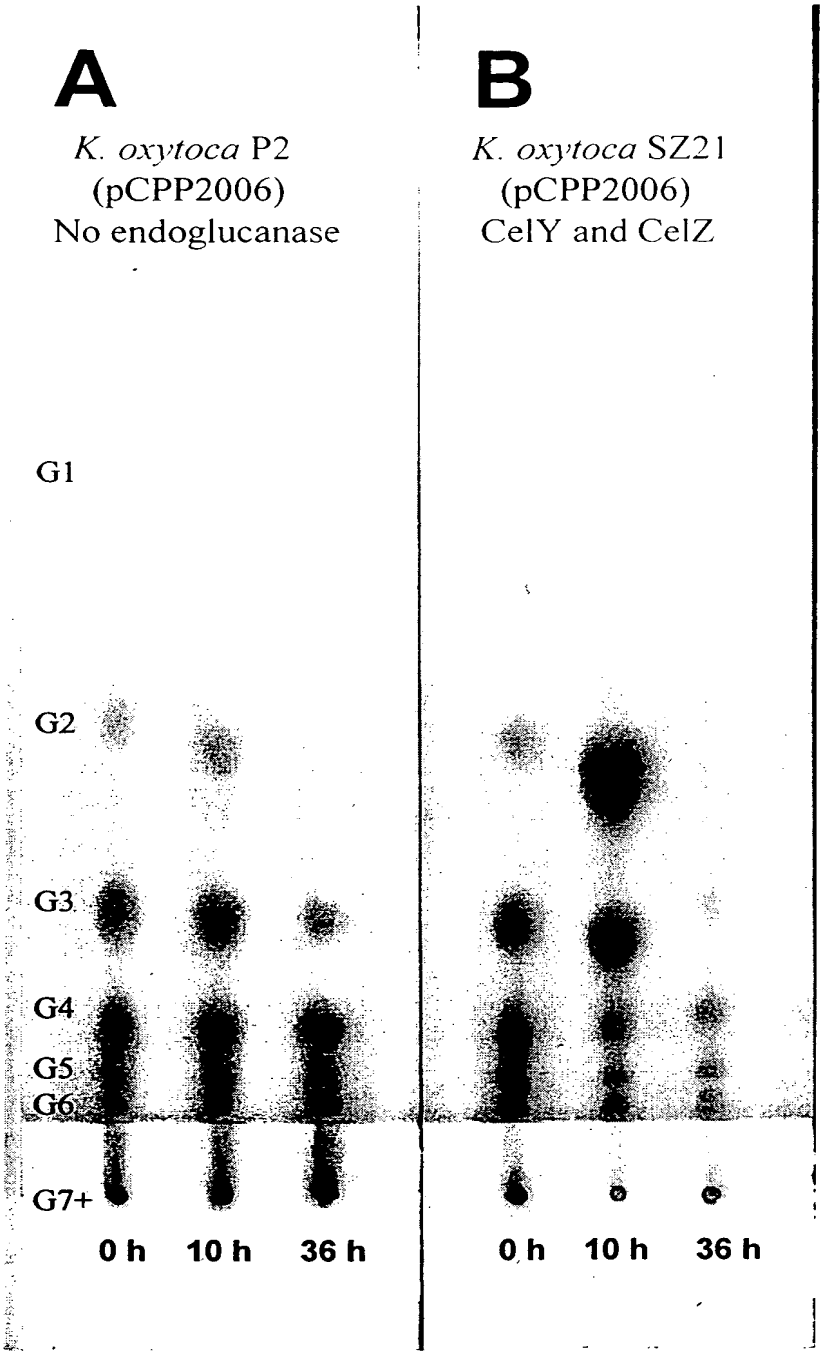
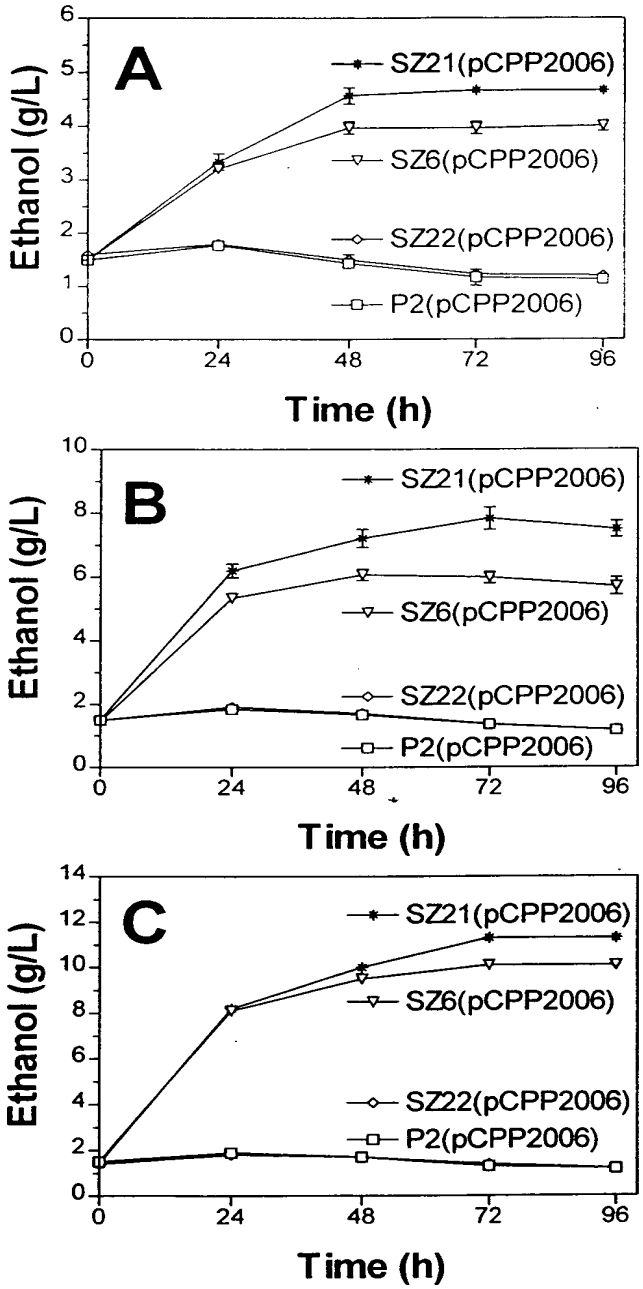

A
K. oxytoca P2
(pCPP2006)
No endoglucanase
B
K. oxytoca SZ21
(pCPP2006)
CelY and CelZ
G1
G2
G3
G4
G5
G6
G7+
0 h
10 h
36 h
0 h
10 h
36 h

Fig. 18


A
SZ21(pCPP2006)
SZ6(pCPP2006)
SZ22(pCPP2006)
P2(pCPP2006)
Ethanol (g/L)
Time (h)
B
SZ21(pCPP2006)
SZ6(pCPP2006)
SZ22(pCPP2006)
P2(pCPP2006)
Ethanol (g/L)
Time (h)
C
SZ21(pCPP2006)
SZ6(pCPP2006)
SZ22(pCPP2006)
P2(pCPP2006)
Ethanol (g/L)
Time (h)